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Development of a rapid method for *Proteus* spp. detection in urine by peptide nucleic acid fluorescence in situ hybridisation (PNA)

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Objective: The need to avoid empirical treatment of patients, and the fact that urine samples are among the most numerous of specimen types sent for microbiology studies, have prompted many researchers to explore methods to limit the time and expense of urine culture processing. The aim of this work was to develop a new peptide nucleic acid fluorescence in situ hybridization (PNA FISH) method for the rapid detection of *Proteus* spp., a genus related to the emergence of complicated urinary tract infections, especially for immuno-compromised patients.

Methods: The PNA probe was designed, optimized, tested on representative strains of the genus and other related strains, and, finally, a PNA FISH method was developed for application in urine samples.

Results: The PNA FISH method was optimized, and laboratory testing on representative strains from the *Proteus* genus and several related bacterial species, showed experimental specificity and sensitivity both of 100% (sensitivity, 95% CI, 81.5 – 100 and specificity, 95% CI, 91.4 - 100). Then, the PNA FISH method was adapted to the detection of *Proteus* in urine. Artificial urine samples were contaminated with decreasing pathogen concentrations and the PNA FISH method was able to detect, in approximately 2 hours, as low as 1×10^4 CFU/mL, a concentration considered indicative of infection for catheter associated urinary tract infections (CAUTI's).

Conclusions: PNA FISH is a very sensitive, specific and rapid method for *Proteus* detection in urine and it could be a reliable alternative to the currently used culture-based techniques as it may avoid the need for empirical antibiotic treatment.
